REMARKS

The applicants are pleased to acknowledge the withdrawal of the rejections and/or objections previously applied.

The specification has been reviewed and the noted trademarks have been identified as such by this Amendment.

The objections to claims 93, 101 and 102 in paragraphs 6 and 7 of the specification have been overcome by this Amendment which has added the term --and-- where appropriate in claims 93 and 102 as well as correcting the spelling of --and-- in claim 102.

In paragraph 9 of the Office Action, claims 93-100 and 102-109 were rejected under 35 U.S.C.§ 1023(b) as being anticipated by Okabayashi et al. (JP HO1-257492) (herein Okabayashi).

Reconsideration is requested.

The inventor named in the Okabayashi patent, is author of the previously cited and withdrawn Okabayashi publication. The applicants have carefully studied the translation of this patent and except for mentioning the temperature 20-37°C, the subject matter of the patent actually corresponds to the subject matter of the publication. (Cell structure and function. Okabayashi et al.; 1989. vol 14, pages 579-586).

The data in the patent and the data in the publication both report the same results after 24 hours of culturing (table at page 10 of the translation of the patent; page 582 of the publication).

The figures reported for incubation times, in page 15 of the patent, correspond to table 2 of page 584 of the publication.

It has to be noted that in the publication, as well as in the patent, reference is always made to 24 hours of incubation in the presence of butyrate. This appears at page 10, lines 7-9; at page 12, lines 10-11 (of the text below the table); at page 13, lines 9-10 (of the text below the table); and at page 16, lanes 4-5.

Moreover, in both the publication and the patent, the incubation time in the presence of butyrate ranges from 6 to 24 hours. Please, refer to the patent at page 14, lines 8-10 (of the text below the table).

In both the patent and the publication the authors (Okabayashi et al.) refer to the fibrin plate method for the detection of the product. In Table V, at page 14, and table VI, page 15 of the patent the same authors identify the product as ProUK.

This shows that they are aware of the fact that the analytical method used is intrinsically responsible for the transformation of the whole proUK in active urokinase. This points out that the analytical method is useful only to detect the total amount of urokinase, without respect to the amount of active two-chain urokinase, which is only produced at a level of 95% according the applicants' claimed invention. In the present application, claim 93, for example, points out that active two-chain urokinase is produced at a level of at least 95%.

The assay used in Okabayashi does not allow a skilled worker in the art to retrieve any further information because the urokinase is detected in the presence of an activator of uPA, as noted at page 582, 1st line:

``...This assay involves the use of an agar plate containing plasminogen and fibrin; when a sample containing a plasminogen activator is added to a well in the agar, the plasminogen is converted to the active enzyme plasmin.''

It is requested that the examiner note that since the assay mixture contains plasminogen, which is the precursor of an activator of urokinase, the plasminogen, which may be converted to plasmin by even small amounts of active urokinase, and:

1) there is no possibility of deriving any information about the amount of catalytically active uPA originally present in the cell culture, as the total amount of sc-uPA is, in any case, converted to tc-uPA during the assay; moreover,

2) any distinction between tc-uPA and sc-uPA is not found within the disclosure of the Okabayashi paper which is rather focused to a method to increase the overall production of uPA, as confirmed by the fact that this increase is measured also at the intracellular level upon an unprocessed protein.

It is important to note that in the patent, Okabayashi only uses the term Prourokinase which corresponds to a specific polypeptide with a peculiar aminoacidic sequence. In the opposite, the use of the term ``urokinase'' is very general, and does not correspond to the HMW tc-uPA or LMW tc-uPA, as claimed in the present application. These materials are each characterized by a specific aminoacid sequence. This fact makes it apparent that anybody skilled in the art reading Okabayashi would not expect the butyrate effect of promoting the maturation of the Pro-UK molecule to 95% pure two chain molecule forms. The only expected product would be

ProUk, or, worse, an undefined mixture of inactive and active, UK-like molecules.

It is important to note that, at the last line of page 14 of the translation of the Okabayashi patent, the authors refer to ProUK:

``the effect of fortifying the generation of proUK by sodium butyrate ... as carried out by using F12 medium alone was defined as a control...''.

At page 22, 2nd and 3rd line, the authors also say:

``...whereas human ProUK can be generated by introducing the DNA into an appropriate recipient cell''

The observation made by the examiner at page 4 of the official action, which referred to the period of ``more than 100 days, exchanging the medium every 2-3 days'' does not anticipate the claims of the present application. The above mentioned period of ``100 days'' actually refers to a time of cultivation where the cells are grown in a medium in the absence of butyrate. The reason for mentioning the period of ``100 days'' in Okabayashi's patent is only to emphasize the stability of the clone. A further reason supporting this interpretation is represented by the fact that butyrate negatively affects the viability of the cell culture as it is widely reported in literature and also experimentally shown in our specification (refer to tables 1, 2, and 3). It should be noted that the viability of a 5 day old culture in the presence of 1.2mM Na-butyrate at 34°C is reduced to 67%. No reference is made in Okabayashi's patent to a period of time longer than 24 hours in the context of incubation when the medium contains butyrate. In fact, in the publication, on page 582, it is reported that:

..We selected two recombinant cell lines, C-68-53 and C-68-61 (the same reported in the patent)...these cell lines appear to be stable since the urokinase production did not change after 8 serial passages in culture (equivalent to 50 cell population doublings)...In these experiments,...cultures of each cell line were incubated for 24 hours either in the presence or in the absence of butyrate, and then the fibrinolytic activity of the conditioned media was measured.

Accordingly, in the patent, at page 25, relative to the selection of the transformed cells (and not to the butyrate treatment) it is reported:

... C-68-53 and C68-61 secreted, into the medium, human UK-like proteins in stable and consistent fashions for more than 100 days.

For the reasons indicated above, the Okabayashi patent does not inherently disclose a cultivation in the presence of butyrate that provides at least 95% of two chain urokinase because the teaching as to the 100 days of culture requires that the medium be exchanged every 3 days The actual cultivations carried out by Okabayashi were for 3 days or 72 hours and only 24 hours after the addition of the butyrate which the Rule 132 Declaration, of record has shown to be ineffective to produce the 95% level of two chain urokinase required by the claims. The doctrine of anticipation by inherency requires exact identity between the claimed invention and the prior art processing conditions in order to establish a *prima facie* case of anticipation. The difference in the concentration of the two chain urokinase and the absence of conditions in the cited reference which the applicant has demonstrated as necessary to obtain a 95% concentration of two chain urokinase establishes that the claimed process is not inherent in the cited prior art. New claim 111 point out the recovery of two chain urokinase

according to page 17, lines 18-22 and Example 4 of the present specification which is not disclosed by Okabayashi. Moreover, claim 102 points out a 120 hour cultivation time and claim 105 recites a serum free process which are not disclosed by Okabayashi. It was not possible, and definitively not obvious, to get any clue from the disclosures of Okabayashi on the solution of the technical problem of: ``processing of the urokinase precursor to obtain 95% pure active product'', that is instead the matter of our application.

In conclusion, Okabayashi does not disclose the combination of the three following characteristics of the claimed invention: Two-chain urokinase, its level of 95% conversion from the Pro UK precursor, and duration of incubation in the presence of butyrate above 24hrs. For these reasons, it is requested that this ground of rejection be withdrawn.

In paragraph 10 of the Office Action, Claims 99 and 108 were rejected under 35 U.S.C.§103(a) as being unpatentable over Okabayashi

According to the Examiner the teaching of Chuppa et al. combined with Okabayashi's renders obvious claims 99 and 108 (temperature from 33°C to 35°C).

However, this rejection is in error for the same reasons set forth above and in view of the fact that Chuppa's teaching is not general, as it refers in particular to perfusion cell fermentors (see the title: ``Fermentor temperature as a tool for control of high-density perfusion cultures of mammalian cells'').

Those who are skilled in the art know that perfusion is a very specific fermentation system for long term culturing of cells (also called " in continuous" cultures) and that i.e. the cell density is higher (typically 15-20 x 10^6) than the one

used in a typical batch culture, one order of magnitude lower (i.e. 1-4 x 10^6).

In addition, it should be noted that Chuppa, at page 338, left column states that: "The effect of reduced growth rate (by the use of lower temperature) on a long term fermentation is minimal". The "long term" referred to in this publication, is in term of months (7 months in fig.11 of the same paper, see also fig. 3 a) and b) - 110 days). Other fermentation systems, such as the batch-culture described in the present Application, deal with few days culture. From the statement of Chuppa it is apparent that short term fermentations such as the one described in the present application, would exhibit a greater decrease in the growth rate due to lower temperature.

In addition and finally the Chuppa reports (see Abstract, l. 9-12) that the outcome in terms of the obtained yields is highly unpredictable and variable from case to case. For these reasons, it is requested that this ground of rejection be withdrawn.

In paragraph 11 of the Office Action, claims 101 and 110 were rejected under 35 U.S.C.§103(a) as being unpatentable over Okabayashi in view of Paques.

Reconsideration is requested.

The Examiner has urged that the step pointed out in claims 101 and 110 is obvious over the combination of Okabayashi and Paques. However, since Paques refers to pasteurization of ``...HMW urokinase as found in urine'', the skilled artisan, aware of the fact that such treatment is useful for natural urokinase as found in urine, would have not applied the same acidification and pasteurization to the product of a recombinant cell culture.

Moreover, the step of filtration in Paques procedure occurs AS THE FINAL STEP OF PURIFICATION. On the contrary the present specification describes filtration of the supernatant, not with the purpose of sterilization (that is a required step for already purified proteins as in Paques), but rather with the purpose of separating the cells from the supernatant containing urokinase. Acidification is made in order to prepare the product to bind to the first column for its INITIAL purification step.

For these reasons, it is requested that this ground of rejection be reconsidered and withdrawn

An early and favorable action is earnestly solicited.

Respectfully submitted,

James V. Costigan
Registration No. 25,669

Hedman & Costigan
1185 Avenue of the Americas
New York, NY 10036
(212) 302-8989

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450 on 9/8/05